The Presence of Mutations in Epidermal Growth Factor Receptor Gene Is Not a Prognostic Factor for Long-Term Outcome after Surgical Resection of Non–Small-Cell Lung Cancer

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Background: The presence of mutation in EGFR gene is known as a predictive marker for the response to epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI) treatment. However, whether or not these EGFR mutations are prognostic factors for non–small-cell lung cancer (NSCLC) is debatable.

Methods: We retrospectively collected a series of samples from patients whose EGFR mutation status had been tested, and analyzed their survival. The pathologic cell types of 863 patients (520 men, 343 women) were squamous cell carcinoma in 227, adenocarcinoma in 636 patients.

Results: EGFR mutations were detected in 354 patients and it was frequently observed in adenocarcinoma in younger, early-stage, female never-smokers. In univariate analysis of younger, early-stage, never-smoker women, bronchioloalveolar carcinoma pattern and the presence of EGFR mutation showed better long-term survival. However, in multivariate analysis, age, pathologic stage, and smoking status remained significant prognostic factors, whereas EGFR mutation was not. For recurrence, pathologic stage was the only independent prognostic factor. After recurrence, smoking status was the only significant risk factor that affected postrecurrence survival. However, when EGFR TKIs were used in EGFR-mutated patients, survival was longer than for those treated with conventional chemotherapy.

Conclusions: Although the EGFR mutation is a predictive marker for EGFR TKI response, it is not a prognostic factor in NSCLC. The clinical observation that patients with EGFR mutation seem to survive longer may be because EGFR mutation is more frequently associated with other good prognostic factors. Once there is a recurrence, administration of EGFR TKI for patients with EGFR mutation may increase survival.

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Recent studies have demonstrated that molecular-targeted agents, such as epidermal growth factor receptor (EGFR) tyrosine kinase inhibitors (TKIs) or anaplastic lymphoma kinase (ALK) inhibitors may prolong survival of selected patients based on tumor biomakers. For example, erlotinib (Tarceva, Astellas Pharma US Inc., NY) demonstrated a survival benefit in unselected non–small-cell lung cancer (NSCLC) patients who previously failed on conventional chemotherapy, but one third of the patients in each arm died by 4 months, and the overall survival advantage was modest.[1] Thus, identifying the patients who will or will not benefit from the EGFR TKIs became crucial for improving patient survival. Some of the molecular biomarkers that have been found useful in predicting the clinical outcomes with EGFR TKIs are EGFR mutations in the tyrosine kinase domain, and these can be detected by DNA sequencing.[2,3] Such molecular biomarkers can predict the outcomes from EGFR TKI treatment and are called predictive markers. However, a prognostic marker is different from a predictive marker, as a prognostic marker is a patient characteristic or a tumor factor that predicts the outcome independent of the treatment administered.

In the study of NSCLC patients treated with chemotherapy with or without EGFR TKIs, the patients with EGFR mutations fared better than those without mutations, irrespective of therapy, which indicates a more indolent biological course of the EGFR-mutated tumors.[4] Thus, the EGFR mutations seem to have a prognostic impact as well. As the prognostic role of a marker is important to take into account when a marker is studied for its prediction of clinical outcome after a given therapy, the prognostic role of the marker should be studied as well. One of the best ways to evaluate the prognostic impact of a marker is to study the patients who underwent surgeries and investigate its role in survival and recurrence. Several articles have reported that the presence of EGFR gene mutations is a prognostic factor.[5–7] However, other reports, including our own previous report, showed no

difference in overall survival between patients with EGFR-mutated tumors and those with wild-type.[8–11] In most of the previous reports, however, the population studied was small and thus, may not have been adequate to control confounding variables, which may have been related to the EGFR mutation and impacted the prognosis.

In this study, we retrospectively investigated the outcomes of the patients who underwent complete resection for NSCLC with reference to EGFR mutational status along with other clinical prognostic variables, in an attempt to ascertain its impact as a prognostic marker for NSCLC.

MATERIALS AND METHODS

Between June 1998 and February 2010, a total of 2440 patients underwent resection for NSCLC at Seoul National University Hospital. Among them, 945 patients were tested for EGFR mutation. We excluded patients with macroscopic or microscopic residual tumors after surgery and those with small-cell carcinoma, large-cell neuroendocrine carcinoma, carcinoids, and other rare pathologic cell types. The final cohort consisted of 863 patients. Preoperative, intraoperative, and postoperative clinical records were reviewed. The routine recording of patient history, findings on physical examination, and results of chest roentgenography was done every 3 months in the first 2 years postoperatively, and twice a year thereafter. Contrast-enhanced chest computed tomography (CT) was done every 6 months for 2 years, then a low-dose chest CT was done twice a year thereafter. An annual examination with positron emission tomography–CT was done simultaneously with chest CT. Conventional adjuvant chemotherapy was recommended for patients with pathologic stage IIa or above. The recurrences were treated based on their pattern, which included surgery, radiation, chemotherapy, or a combination. The EGFR TKIs were recommended as the second-line palliative treatment. Final outcome was recorded at either the time of death or recurrence. Patients who had not visited the clinic were contacted by telephone. Recommendations of the Declaration of Helsinki for biomedical research involving human subjects were also followed. Before January 2005, written informed consent for molecular analysis of surgical specimen was exempted. Written informed consent was obtained from individual patients thereafter, and the study protocol as well as ethical issues were reviewed and approved by Seoul National University Hospital Institutional Review Board (H-1012-130-346/C-1111-102-387).

DNA was extracted from five 10-μm-thick paraffin sections, containing a representative portion of tumor tissue. DNA extraction from formalin-fixed paraffin-embedded tissue was carried out, using GentraPuregene DNA purification kit (Qiagen, Hilden, Germany) according to the manufacturer's protocol. Fifty nanograms of DNA were amplified in a 20-μl reaction solution containing 10 μl of 2× concentrated HotStarTaq Master Mix (Qiagen), including polymerase chain reaction (PCR) buffer, 3 mM MgCl2, 400 μM each of deoxyribonucleotide triphosphate (dNTP), and 0.3 μM each of primer pairs (exon 18F:5′-TCCAAATGAGCTGGCAAGTG-3′, 18R:5′-TCCAAATGAGCTGGCAAGTG-3′; exon 19F:5′-GCAATATCAGCCTTAGGTGCGGCT-3′, 19R:5′-CATAGAAAGTGAACATTTAGGATGTG-3′; exon 20F:5′-CCATGAGTACGTATTTTGAAACTC-3′, 20R:5′-CATATCCCCATGGCAAACTCTTGC-3′; exon 21F:5′-GCTCAGAGCCTGGCATGAA-3′, 21R:5′-CATCCTCCCCTGCATGTGT-3′). Of the first PCR product, 1 μl was proceeded for the nested PCR using each of primer pairs (exon 18F:5′-TGGCAAGTGCCGTGTCCTGGCA-3′, 18R:5′-CTCAGTGAAACAAAGAGTAAAGTAG-3′; exon 19F:5′-CCTTAGGTGCGGCTCCACAGC-3′, 19R:5′-CATTTAGGATGTGGAGATGAGC-3′; exon 20F:5′-GAAACTCAACATCGCATTCATGC-3′, 20R:5′-GCAAACTCTTGCTATCCCAGGAG-3′; exon 21F:5′-CTGGCATGAACATGACCCTG-3′, 21R:5′-TGCATGTGTTAAACAATACAGC-3′). Amplifications of EGFR (exon 18–21) were performed using a 10 minute (min) initial denaturation at 94°C followed by 25 cycles of 60 seconds (s) at 94°C, 60 s at 55°C, and 60 s at 72°C, and a 10-min final extension at 72°C. PCR products were then purified using a 2% gel with a QIAgen gel extraction kit (Qiagen). Nucleic acid (NA) templates were processed for the DNA sequencing reaction using the ABI-PRISM BigDye Terminator version 3.1 (Applied Biosystems, Foster, CA) with both forward and reverse sequence-specific primers. Twenty nanograms of purified PCR products were used in a 10-μl sequencing reaction solution containing 1 μl of BigDye Terminator version 3.1 and 0.1 μM of the same PCR primer. Sequencing reactions were performed using 25 cycles of 10 s at 96°C, 5 s at 50°C, and 4 min at 60°C; sequence data were generated with the ABI-PRISM 3100 DNA Analyzer (Applied Biosystems), and sequences were analyzed by Sequencing Analysis 5.1.1. software (Applied Biosystems) to compare variations.

The relationship between the clinical outcomes (overall survival and freedom from recurrence) and mutation status of EGFR was analyzed along with clinical variables. For the analysis, we selected the following clinical variables that had been known to affect long-term survival of NSCLC: sex, age, smoking status, tumor, node, metastasis stage (according to the 7th edition of American Joint Committee on Cancer staging), and microscopic characteristics. Frequency of gene mutations was calculated for each category and then tested for association by using the χ^2 test for each clinical factor. Overall survival and recurrence were investigated by using the Kaplan–Meier method, and the difference between groups determined by risk factors was tested by using the log-rank test. Cox's proportional hazard model was used to explore the influence of independent prognostic factors in a multivariable model. The factors were chosen by a stepwise-forward method with criteria for variable inclusion of 0.05 and for variable exclusion of 0.10. A 5% significance level was considered for statistical significance. All analyses were performed using IBM SPSS Statistics 19 for Windows (SPSS Inc., Chicago, IL).

RESULTS

The clinical characteristics of the 863 patients are summarized in the Table 1. The mean age was 63 years (range, 27–87). Five hundred and twenty patients were men and 343 were women. Pathologic cell types were squamous cell (SQ) in 227, adenocarcinoma (AD) in 587,

TABLE 1. Clinical features and their relationship with EGFR mutation in All patients

Features	EGFR Mutation		
	Absent	Present	*p*
Sex			0.000
Male	379 (72.9%)	141 (27.1%)	
Female	130 (37.9%)	213 (62.1%)	
Age, yrs	63.9±9.7	61.8±9.7	0.002
Smoking			0.000
Never	149 (37.9%)	244 (62.1%)	
Ever	356 (76.7%)	108 (23.3%)	
Cell type			0.000
Squamous	219 (96.5%)	8 (3.5%)	
Adenocarcinoma	290 (45.6%)	346 (54.4%)	
BAC pattern			0.000
Absent	403 (74.8%)	136 (25.2%)	
Present	106 (32.7%)	218 (67.8%)	
Pathologic stage			0.000
I	266 (54.0%)	227 (46.0%)	
I	105 (75.5%)	34 (24.5%)	
III	114 (60.3%)	75 (39.7%)	
IV	24 (57.1%)	18 (17.2%)	

BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor.

TABLE 2. Univariate and Multivariate Analyses for Overall Survival in all Patients

Variables	Univariate Analysis[a]	Multivariate Analysis[b]	
	p	Hazard Ratio (CI)	*p*
Sex (female)	0.000		
Age yrs	0.005	1.037 (1.014–1.061)	0.002
Smoking (never)	0.000	2.374 (1.496–3.769)	0.000
Cell type (adenocarcinoma)	0.000		
BAC pattern (present)	0.000		
Pathologic stage (I)	0.000		0.000
II		2.389 (1.340–4.259)	0.003
III		3.299 (1.964–5.541)	0.000
IV		6.374 (3.040–13.366)	0.000
EGFR mutation (present)	0.001		
exon 18	0.562		
exon 19	0.037		
exon 20	0.443		
exon 21	0.006		

[a]Kaplan–Meier's survival analysis with log-rank test.
[b]Cox's proportional hazard model.
BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; CI, confidence interval.

adenosquamous cell (ADSQ) in 19, and bronchioloalveolar carcinoma (BAC) in 30 patients. Microscopically, 324 patients showed bronchioloalveolar cell carcinoma components. Four hundred and ninety-three patients (57.1%) were stage I, 393 patients (45.9%) were never-smokers, 305 (35.6%) were exsmokers, and 159 (18.6%) were current smokers. In six patients, smoking history was not available. Neoadjuvant chemotherapy was administered in 23 patients and concurrent neoadjuvant chemoradiation was performed in three patients. None of the patients had been treated with EGFR TKIs before surgery.

EGFR mutations were detected in 354 patients. The most common site of mutation was in exon 19 (exon 18 in 15, exon 19 in 169, exon 20 in 23 and exon 21 in 156 patients). The correlation between the frequency of EGFR mutation and the clinical variables are listed in Table 1. The EGFR mutations were frequently observed in younger, early-stage, female never-smokers. The pathologic cell types were related to the frequency of EGFR mutations. The EGFR mutations were detected more frequently in AD, ADSQ carcinoma, or BAC, whereas they were not frequent in SQ type (SQ 3.5%; AD 54.3%; ADSQ, 57.9%; BAC, 53.3%). We merged AD, ADSQ, and BAC as an AD group and used it for further analysis.

There were eight cases of operative death. Long-term outcome was analyzed for 855 patients, excluding the eight operative death cases. During the follow-up period (23.6±0.7 months), 235 patients (27.5%) experienced recurrences and 66 patients (28.1%) died of the disease. Among the patients who did not recur, 36 patients (4.2%) died of various causes not related to their lung cancer. The overall 5-year survival of the 855 patients was 72.9±3.7%. The 5-year freedom from recurrence rate was 26.9±5.0%.

The overall survival was analyzed based on the clinical variables. In univariate analysis, in the younger age, early-stage, never-smoker women, the presence of BAC components and the presence of EGFR mutation showed better long-term survival (Table 2, Fig. 1). As there were tight correlations between the presence of EGFR mutation and good clinical variables, we performed multivariate analysis to correct hidden confounding effects. In Cox's proportional hazard model, age, pathologic stage, and smoking status remained as significant prognostic factors, whereas EGFR mutation status was not (Table 2). We used the same analysis for AD patients. Similar results were observed, and the only difference was that the age was not included as a significant variable in the multivariate analysis for overall survival (Table 3).

We also analyzed factors associated with the development of recurrence. In univariate analysis, earlier pathologic stage ($p = 0.000$) and the presence of BAC pattern ($p = 0.008$) showed better outcome in terms of recurrence. The presence of EGFR mutation was not related to the occurrence of recurrence. In multivariate analysis, pathologic stage ($p = 0.000$) remained the only independent factor that affected recurrence (Table 4). The results were the same when we analyzed the patients with AD (Table 5).

As there was no difference in the development of recurrence according to the EGFR mutation status, we suspected that the survival difference was a result of a difference in postrecurrence survival and subsequently, analyzed 235 patients who recurred. The female ($p = 0.021$) never-smoker ($p = 0.009$) survived longer after recurrence. The cell type ($p = 0.485$), BAC pattern ($p = 0.149$), and pathologic stage ($p = 0.300$) were not significant. Although

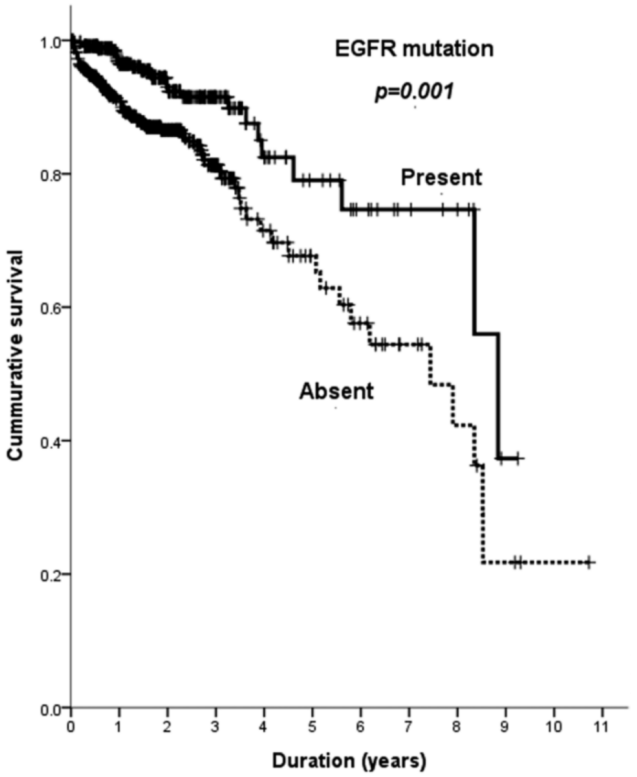

FIGURE 1. Comparison of survival curve after curative resection of NSCLC according to the presence of EGFR mutations (Kaplan–Meier's survival curve with log-rank test). NSCLC, non–small-cell lung cancer; EGFR, epidermal growth factor receptor.

TABLE 3. Univariate and Multivariate Analyses for Overall Survival in Patients with Adenocarcinoma

	Univariate Analysis[a]	Multivariate Analysis[b]	
Variables	***p***	**Hazard Ratio (CI)**	***p***
Sex (female)	0.005		
Age yrs	0.331		
Smoking (never)	0.002	2.482 (1.451–4.244)	0.001
BAC pattern (present)	0.002		
Pathologic stage (I)	0.000		0.001
II		1.326 (0.574–3.065)	0.509
III		2.309 (1.248–4.273)	0.008
IV		4.621 (2.005–10.646)	0.000
EGFR mutation (present)	0.012		
exon 18	0.286		
exon 19	0.210		
exon 20	0.194		
exon 21	0.010		

[a]Kaplan–Meier's survival analysis with log-rank test.
[b]Cox's proportional hazard model.
BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; CI, confidence interval.

TABLE 4. Univariate and Multivariate Analyses for Freedom from Recurrence in all Patients

	Univariate Analysis[a]	Multivariate Analysis[b]	
Variables	***p***	**Hazard Ratio (CI)**	***p***
Sex (female)	0.707		
Age yrs	0.408		
Smoking (never)	0.504		
Cell type (adenocarcinoma)	0.951		
BAC pattern (present)	0.008		
Pathologic Stage (I)	0.000		0.000
II		2.019 (1.376–2.962)	0.000
III		2.351 (1.705–3.241)	0.000
IV		6.549 (4.076–10.523)	0.000
EGFR mutation (present)	0.959		
exon 18	0.308		
exon 19	0.822		
exon 20	0.351		
exon 21	0.880		

[a]Kaplan–Meier's survival analysis with log-rank test.
[b]Cox's proportional hazard model.
BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; CI, confidence interval.

there was a tendency of better survival in the EGFR mutation group ($p = 0.061$), it did not reach statistical significance (Fig. 2). In multivariate analysis, smoking status ($p = 0.008$, hazard ratio=2.152 [1.223–3.788]) remained the only factor that affected postrecurrence survival. Among the 235 recurred patients, 97 patients were harboring EGFR mutations. In the EGFR mutation group (97), 56 patients were treated with EGFR TKIs as a second-line treatment after failure of conventional chemotherapy, 10 patients were treated with conventional chemotherapy, one patient with crizotinib, (another kind of target agent and an ALK inhibitor), and the remaining 30 patients did not receive systemic chemotherapy and were treated with either local modality or supportive care. In wild-type EGFR group (138), 30 received EGFR TKIs, 49 received conventional chemotherapeutic agents, and 59 received local or best supportive care. We stratified the recurred patients based on the EGFR mutation status and tested postrecurrence survival with regard to the systemic treatment given. In wild-type EGFR group, there was no difference in each treatment modality. However, in the EGFR mutation group, the survival was different ($p = 0.020$) and patients who were treated with EGFR TKI had the best survival rate, followed by the local treatment or best supportive care group. Patients who were treated with conventional chemotherapy showed a poor prognosis (Fig. 3). The difference between EGFR TKI and conventional chemotherapy groups was statistically significant ($p = 0.001$).

DISCUSSION

A prognostic marker is a patient characteristic or a tumor factor that predicts the outcome independent of treatment administered, and a predictive marker is a clinical or molecular

TABLE 5. Univariate and Multivariate Analyses for Freedom from Recurrence in the Patients with Adenocarcinoma

Variables	Univariate Analysis[a]	Multivariate Analysis[b]	
	p	Hazard Ratio (CI)	*p*
Sex (female)	0.540		
Age yrs	0.529		
Smoking (never)	0.244		
BAC pattern (present)	0.002		
Pathologic stage (I)	0.000		0.000
II		2.333 (1.462–3.723)	0.000
III		2.488 (1.736–3.565)	0.000
IV		6.929 (4.164–11.531)	0.000
EGFR mutation (present)	0.810		
exon 18	0.263		
exon 19	0.692		
exon 20	0.392		
exon 21	0.520		

[a]Kaplan–Meier's survival analysis with log-rank test.
[b]Cox's proportional hazard model.
BAC, bronchioloalveolar carcinoma; EGFR, epidermal growth factor receptor; CI, confidence interval.

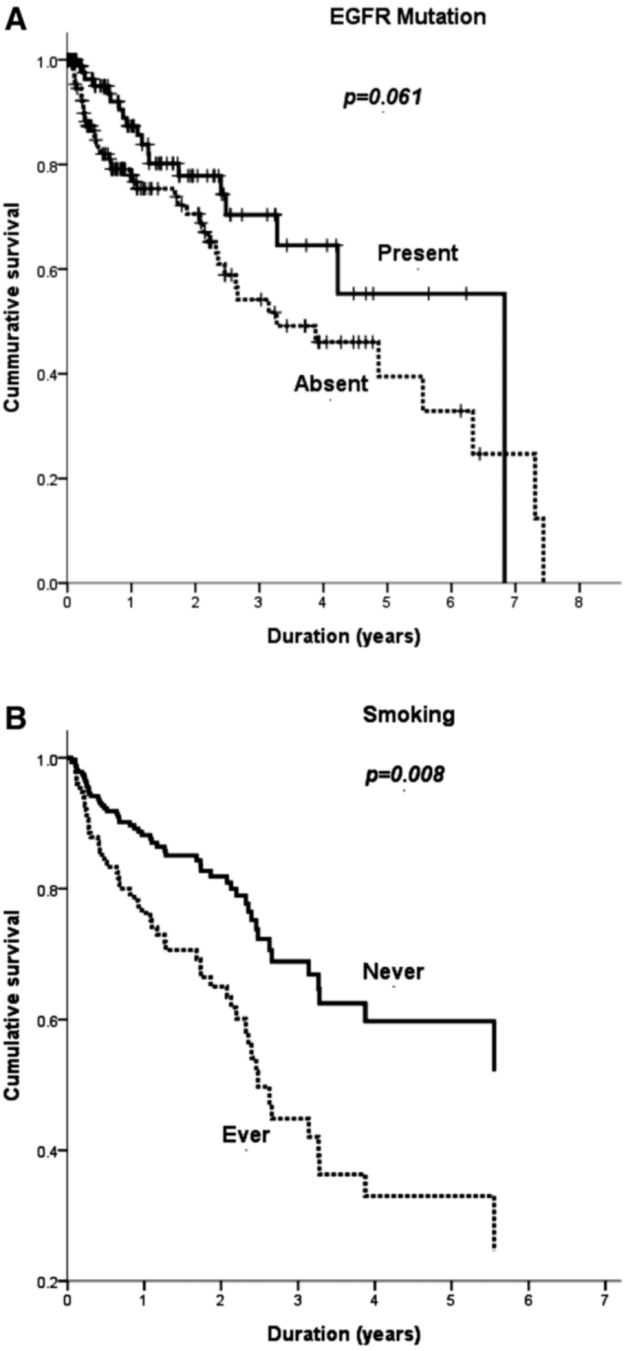

FIGURE 2. Comparison of survival curve after recurrence according to the presence of EGFR mutations (A), Kaplan–Meier's survival curve with log-rank test and smoking status (B), Cox's proportional hazard model. EGFR, epidermal growth factor receptor.

marker that predicts the outcome of a specific treatment. The most typical example is human epidermal growth factor receptor 2 in breast cancer, which is an unfavorable prognostic marker in the patients treated only with surgery, but a good predictive marker for treatment with trastuzumab (Herceptin, Roche Ltd., Basel, Switzerland).[12] The discovery of specific EGFR mutations associated with sensitivity to EGFR TKIs was significant and provided new insights into the mechanisms of the sensitivity to these drugs.

Although there is consistency in the data showing a strong association between specific EGFR mutations and response to EGFR TKIs in advanced NSCLC, the role of EGFR mutations as a prognostic marker for survival in patients with NSCLC is still not proven. The Iressa NSCLC Trial Assessing Combination Treatment (INTACT) study did show a significantly increased survival of EGFR mutation-positive patients treated with chemotherapy, irrespective of EGFR TKI.[13] These findings are also in agreement with the molecular analysis of a phase III trial of erlotinib (Tarceva responses in conjunction with paclitaxel and carboplatin [TRIBUTE]).[4] Others, however, have reported no prognostic role.[9–11,14] Thus, the role of EGFR mutations as a prognostic marker in NSCLC remains controversial. The discrepancies in these studies may be because of the existence of confounding factors such as different clinical characteristics.

Previously, we reviewed 71 lung AD patients who underwent surgical resection and analyzed the presence of EGFR mutations as a prognostic factor for long-term survival.[10] In that study, we demonstrated that no association existed between EGFR mutation and overall survival after we had controlled confounding variables. Similar reports have been subsequently published by many centers.[5,15,16] Compared with previous reports, our current series is one of the largest studies that tested the value of EGFR mutation in patients after surgery as a prognostic marker. The frequency of EGFR mutation in our series was 41%, and if we selected only AD patients, it would have been 54.3%. The prevalence of EGFR mutation is reported to be higher in East Asians compared with whites.[17] With an increasing number of female, nonsmoking lung cancer patients, the frequency of detecting EGFR mutations seems to

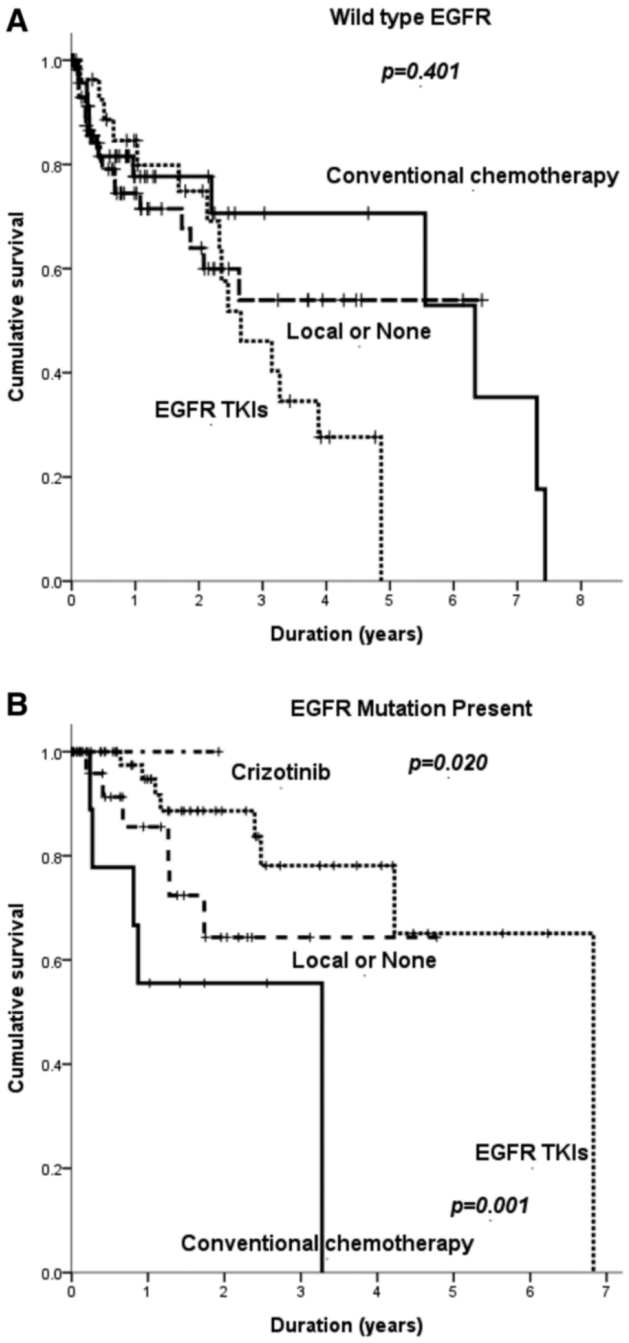

FIGURE 3. Comparison of survival curve after recurrence according to the presence of EGFR mutations and palliative treatment modalities (Kaplan–Meier's survival curve with log-rank test) (A), wild-type EGFR, (B), mutated EGFR.EGFR, epidermal growth factor receptor.

be increasing in recent years. It is noteworthy that the frequency of EGFR mutations among the patients with AD in our hospital during the recent 1-year period was as high as 58.0% (data not published). In our previous study, we were not able to find a statistically significant association between sex and EGFR mutation, and suggested that a larger study population might have resulted in statistical significance. In our current study, using a larger number of patients, we could successfully demonstrate that EGFR mutation was more prevalent in women than in men. Other clinical variables such as age, stage, cell type, smoking status, and the presence of BAC pattern were associated with EGFR mutation. Such observations coincided with the results of others.[5] In univariate analysis, the long-term survival was better in younger, early-stage, never-smoker women, and also when there was a BAC pattern along with the presence of EGFR mutations. As these clinical variables are already well-known prognostic factors, the good prognostic outcome in patients with EGFR mutations may be because of the effect from the close linking of these variables to the presence of EGFR mutations (Table 1). Hence, it is not easy to investigate the true prognostic role of EGFR mutation. To control the confounding effects of these clinical prognostic factors, we performed multivariate analysis using Cox's proportional hazard model. In the final model, age, pathologic stage, and smoking status remained the significant prognostic factors for overall survival, whereas EGFR mutation status was not. This result suggests that the presence of EGFR mutation itself may not be a true prognostic factor for long-term survival in NSCLC. Similar results have been demonstrated by others.[14–16,18]

As the NSCLC has heterogenous cell types and the EGFR mutation is rarely detected in SQ carcinoma, we performed the same analysis excluding SQ carcinoma. The correlation between clinical variables and the presence of EGFR mutations was similar to that of entire cell types except for age. However, as the difference of the age in the entire group was only 1 year it seemed to have no meaning from a practical point of view. Multivariate analysis resulted in similar observations except for the fact that age was omitted from the final model. Only pathologic stage and smoking status remained statistically significant variables (Table 3).

Although the current study is based on a large number of patients, most of them were in early stage. As a consequence, the number of recurrences and subsequent cancer-specific mortalities were relatively low. The mean follow-up duration of 23.6 months may not be sufficient to come to a definite conclusion. However, our result can add the evidence that EGFR mutation status alone is not a meaningful prognostic biomarker for early-stage NSCLC.

We analyzed whether or not the EGFR mutation status affected the development of recurrence. The presence of EGFR mutations was not a significant prognostic factor for either disease-free survival (log-rank test $p = 0.148$) or freedom from recurrence (log-rank test $p = 0.959$, Fig. 4). If the EGFR mutation was related to the tumor behavior and happened to be a prognostic factor, the recurrence should have been affected by the presence of EGFR mutation. Such observations of recurrence coincides with the results of Sonobe et al.[6] They selected 32 KRAS mutation patients and 48 EGFR mutation patients and compared the disease-free survival rates. They found that disease-free survival was influenced by the mode of surgical resection and the pathologic stage of the lung cancer. Age, sex, smoking status, and gene mutations were not significant. Interestingly, however, only pathologic stage and gene mutations were the significant factors for determining

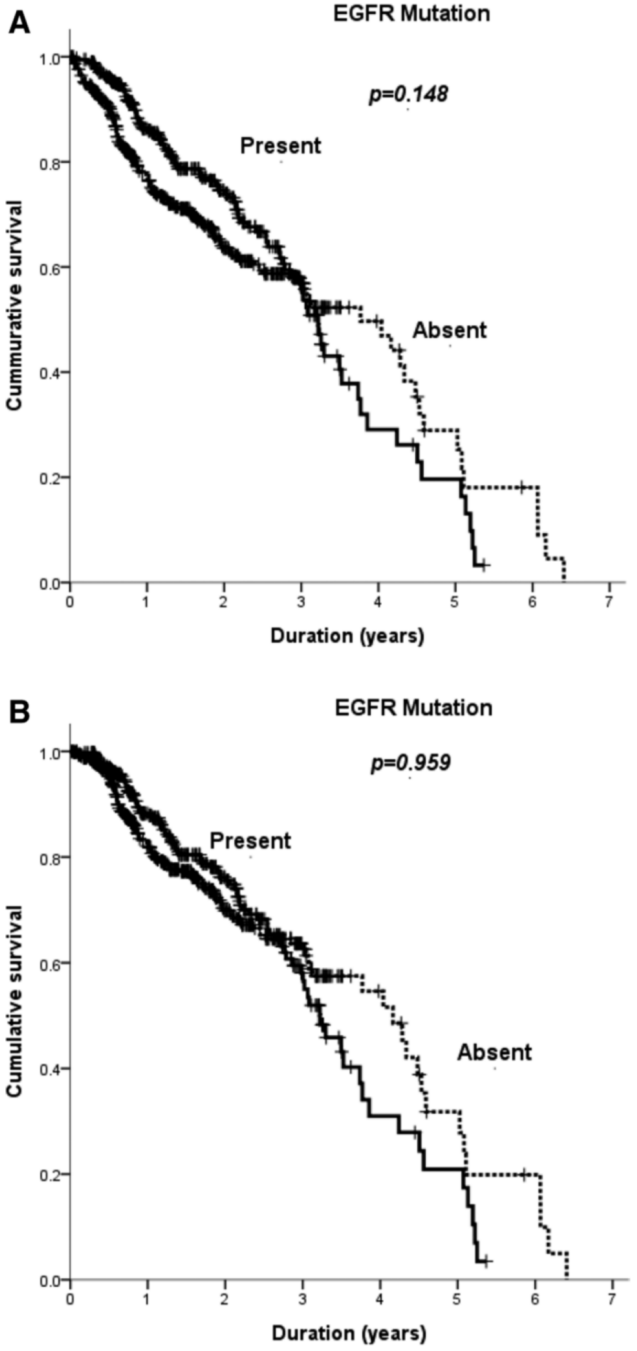

FIGURE 4. Comparison of disease-free survival (A), and freedom from recurrence (B), according to the presence of EGFR mutations (Kaplan–Meier's survival curve with log-rank test). EGFR, epidermal growth factor receptor.

overall and long-term survival. Their finding is different from our study, where the patients with EGFR mutations showed better overall survival than those without in univariate analysis only (Fig. 1). In multivariate analysis, the EGFR mutation was no longer a significant prognostic factor (Table 1). The role of KRAS mutation as a prognostic marker may explain the discrepancy. Their comparison was between EGFR-mutated patients, and KRAS-mutated patients, and excluded those with both the wild-type. We have previously reported that the KRAS mutation was a poor prognostic factor in AD of the lung.[10] As the KRAS mutation is well known to be a poor prognostic marker, their observation of better overall survival in the EGFR mutation group could have resulted from a poor overall survival of the KRAS mutation group. Unfortunately, the KRAS mutations were not tested in our patient cohort and we could not prove this issue in this study. They also analyzed survival after recurrence, and treatment modality, and suggested that a better responsiveness to EGFR TKIs might explain the superior survival rate in the EGFR mutation group.[6] When we compared postrecurrence survival in both the groups, the EGFR mutation patients showed a trend for better survival. However, the *p* value did not reach statistical significance (Fig. 2). In multivariate analysis, smoking status ($p = 0.008$, hazard ratio=2.152 [1.223–3.788]) remained the only factor which affected postrecurrence survival and the EGFR mutation was not. We also tested to determine whether or not there is any survival benefit of using EGFR TKIs for patients with EGFR mutations. We stratified recurred patients based on the EGFR mutation status and the treatment given. In wild-type EGFR group, there was no difference in each treatment modality. By contrast, for those with EGFR mutation, the survival was different ($p = 0.020$), and patients who were treated with EGFR TKIs survived longer than those treated with conventional chemotherapeutic regimens ($p = 0.001$, Fig. 3). Such observations coincided with our current knowledge that EGFR mutation is a predictive marker for EGFR TKI treatment.

In conclusion, although the EGFR mutation is a predictive marker for EGFR TKI response, the presence of EGFR mutation is not a prognostic factor in NSCLC. The clinical observation that patients with EGFR mutation seem to survive longer may be because EGFR mutation is more frequently associated with other significant prognostic factors, such as age, stage, or smoking status. On the basis of our results, the pathologic stage seems to affect the prognosis by influencing the cancer recurrence, and smoking status seems to be the most important prognostic factor for overall survival. For recurred patients, administration of EGFR TKI for EGFR-mutated patients may offer improved survival gain over those who receive conventional chemotherapy.

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REFERENCES

1. Shepherd FA, Rodrigues Pereira J, Ciuleanu T, et al.; National Cancer Institute of Canada Clinical Trials Group. Erlotinib in previously treated non-small-cell lung cancer. *N Engl J Med* 2005;353:123–132.
2. Lynch TJ, Bell DW, Sordella R, et al. Activating mutations in the epidermal growth factor receptor underlying responsiveness of non-small-cell lung cancer to gefitinib. *N Engl J Med* 2004;350:2129–2139.

3. Paez JG, Jänne PA, Lee JC, et al. EGFR mutations in lung cancer: correlation with clinical response to gefitinib therapy. *Science* 2004; 304:1497–1500.
4. Eberhard DA, Johnson BE, Amler LC, et al. Mutations in the epidermal growth factor receptor and in KRAS are predictive and prognostic indicators in patients with non-small-cell lung cancer treated with chemotherapy alone and in combination with erlotinib. *J Clin Oncol* 2005;23:5900–5909.
5. Marks JL, Broderick S, Zhou Q, et al. Prognostic and therapeutic implications of EGFR and KRAS mutations in resected lung adenocarcinoma. *J Thorac Oncol* 2008;3(2):111–116.
6. Sonobe M, Kobayashi M, Ishikawa M, et al. Impact of KRAS and EGFR gene mutations on recurrence and survival in patients with surgically resected lung adenocarcinomas. *Ann Surg Oncol* 2012; 19 Suppl 3:S347–S354.
7. Sonobe M, Nakagawa M, Takenaka K, et al. Influence of epidermal growth factor receptor (EGFR) gene mutations on the expression of EGFR, phosphoryl-*Akt*, and phosphoryl-*MAPK*, and on the prognosis of patients with non-small cell lung cancer. *J Surg Oncol* 2007;95:63–69.
8. Shigematsu H, Lin L, Takahashi T, et al. Clinical and biological features associated with epidermal growth factor receptor gene mutations in lung cancers. *J Natl Cancer Inst* 2005;97:339–346.
9. Tokumo M, Toyooka S, Kiura K, et al. The relationship between epidermal growth factor receptor mutations and clinicopathologic features in non-small cell lung cancers. *Clin Cancer Res* 2005;11:1167–1173.
10. Kim YT, Kim TY, Lee DS, et al. Molecular changes of epidermal growth factor receptor (EGFR) and KRAS and their impact on the clinical outcomes in surgically resected adenocarcinoma of the lung. *Lung Cancer* 2008;59:111–118.
11. Kim ST, Choi YJ, Park KH, et al. Epidermal growth factor receptor mutations as a prognostic factor in Korean patients with advanced lung adenocarcinoma who had not been treated with received epidermal growth factor receptor tyrosine kinase inhibitors. *Chemotherapy* 2011;57:108–114.
12. Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL. Human breast cancer: correlation of relapse and survival with amplification of the *HER-2/neu* oncogene. *Science* 1987;235:177–182.
13. Bell DW, Lynch TJ, Haserlat SM, et al. Epidermal growth factor receptor mutations and gene amplification in non-small-cell lung cancer: molecular analysis of the IDEAL/INTACT gefitinib trials. *J Clin Oncol* 2005;23:8081–8092.
14. Sasaki H, Shimizu S, Endo K, et al. EGFR and *erbB2* mutation status in Japanese lung cancer patients. *Int J Cancer* 2006;118: 180–184.
15. Kosaka T, Yatabe Y, Onozato R, Kuwano H, Mitsudomi T. Prognostic implication of EGFR, KRAS, and *TP53* gene mutations in a large cohort of Japanese patients with surgically treated lung adenocarcinoma. *J Thorac Oncol* 2009;4:22–29.
16. Lim KH, Huang MJ, Liu HC, Kuo HT, Tzen CY, Hsieh RK. Lack of prognostic value of EGFR mutations in primary resected non-small cell lung cancer. *Med Oncol* 2007;24:388–393.
17. Chan SK, Gullick WJ, Hill ME. Mutations of the epidermal growth factor receptor in non-small cell lung cancer – search and destroy. *Eur J Cancer* 2006;42:17–23.
18. Kobayashi N, Toyooka S, Ichimura K, et al. Non-BAC component but not epidermal growth factor receptor gene mutation is associated with poor outcomes in small adenocarcinoma of the lung. *J Thorac Oncol* 2008;3:704–710.